## REMARKS

Applicants have received and reviewed the Office Action dated July 31, 2001. By way of response, Applicants have cancelled claims 12, 13, 16 and 17 without prejudice, amended claims 1, 14 and 18, and added claims 20-24. Claims 1-11, 14, 15 and 18-24 are pending. No new matter is introduced. Applicants submit that the amended and newly presented claims are supported by the specification.

In particular, support for the recitation in the claims regarding an assay can be found in the specification at least at pages 15-16. Support for treating a patient according to the invention can be found in the specification at least at pages 11-15 and 26-28.

For the reasons given below, Applicants respectfully submit the amended and newly presented claims are in condition for allowance, and notification to that effect is earnestly solicited.

### Objections to the Specification and Drawings

Applicants thank Examiner for pointing out the typographic errors in the specification and drawings. In response, Applicants have made amendments to correct these minor errors.

### Rejections under 35 U.S.C. § 101

The Examiner has rejected claims 12, 13 and 16 under 35 U.S.C. § 101 for reciting a use without setting forth any steps in a process. In response, Applicants have cancelled claims 12, 13 and 16 without prejudice. New claims 20-23 recite the subject matter of cancelled claims 12 and 13 in appropriate dependent format and replace the objectionable claim language with appropriate method claim language. New claim 24 recites an assay for a substance and the appropriate steps for practicing the assay. As mentioned above, support for claim 24 can be found in the specification at least at pages 15-16.

For these reasons, Applicants respectfully submit that the claims comply with 35 U.S.C. § 101 and notification to that effect is earnestly solicited.

### Rejections under 35 U.S.C. § 112, first paragraph

The Examiner has rejected claims 12-15 and 1-11 under 35 U.S.C. § 112, first paragraph, as containing subject matter not enabled by the specification. Although this rejection has not

been raised for the newly presented claims, it is discussed insofar as it might be applied. Applicants respectfully traverse this rejection.

According to the MPEP, the test of enablement is "whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without any undue experimentation." MPEP 2164.01 (citing United States v. Telectronics, Inc., 857 F.2d 778, 785 (Fed. Cir. 1988)). The MPEP also indicates that the Examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. MPEP 2164.04. As discussed below, the Examiner has cited the Wands factors, but has failed to provide objective evidence that the claims are not enabled. To the contrary, the specification in fact meets the standard of enablement for the full scope of the claims.

With regard to therapeutic applications, on page 5 of the Office Action the Examiner states "the present claims are very broad." The Examiner so characterizes Claims 12 and 14, but does not provide objective evidence that the disclosure does not support the full breadth of these claims. The Examiner characterizes the relevant aspects of the invention as "gene therapy" and as "transplantation of untransformed neural tissue" (pages 5-6). Similarly, with regard to the type of precursor cell used, on page 8 of the Office Action, the Examiner states "the present claims are very broad," asserting that claim 1 encompasses proliferating and differentiating any precursor cells.

The Rejection of claims 12-15

The Examiner has characterized claims 14-15 as encompassing "gene therapy." Citing Verma, et al. and Palu, et al., the Examiner concludes that gene therapy is a "technique of the future," with no successful outcome documented as of the filing date of the application. In response, Applicants respectfully assert that the Verma et al. (Nature (1997) 389:239-42), Palu et al. (J. Biotechnol. (1999) 68:1-13) and Fox (ASM News (Feb. 2000) 66(2):1-3) references are not relevant. Although the references discuss gene therapy in general, the Application is directed more specifically to *ex vivo* production of viable dopaminergic neurons for the treatment of neurological disorders, which were not heretofore available or producible in sufficient numbers. Furthermore, the application provides a method of treating neurological disorders that combines

particular disorder, will have more than reasonable guidance in practicing the method as claimed without undue experimentation.

The Examiner notes a lack of working example wherein transformed cells prepared according to the invention are injected into an animal (page 7). However, as discussed in the MPEP at 2164.02, the specification need not contain an example if the invention is otherwise disclosed in such a manner that one of skill in the art will be able to practice the invention without an undue amount of experimentation. As argued above, Applicants urge that the invention is indeed described in such a manner. The specification at least at pages 5-11 contains detailed information regarding the preparation of dopaminergic neurons. One skilled in the art would be more than capable of transforming these prepared cells with a gene of interest.

Transplantation of neural cells is also known within the art. The Office Action does not provide any objective evidence that claims 12-13, pertaining to treatment of a patient with neuronal cells cultured according to the invention are not enabled by the specification, and has therefore failed to meet the PTO's burden in this respect. The Examiner, without citing evidence, asserts that there are no documented successes and that neural tissue transplantation is unpredictable. Applicants respectfully disagree with the Examiner in this regard. For example, the specification at page 1, lines 19-24 notes that more than 200 patients have been transplanted with fetal nigral cells worldwide, with confirmed clinical improvement. Olanow, et al., attached hereto, report clinical benefit observed in patients receiving transplants, in addition to robust graft survival and striatal reinnervation. Abstract, Olanow et al., "Fetal nigral transplantation as a therapy for Parkinson's disease," TINS, Vol. 9, No. 3, 1996. Furthermore, Example 5 of the specification demonstrates, contrary to the Examiner's assertions, that the therapeutic effect of treatment can be sustained. For example, on page 26, lines 25-29 of the specification, it is noted that 80 days post-transplantation, a substantial improvement in rotation scores could be detected in 5 out of 7 animals, with no improvement noted in controls. The specification further notes the Applicants' demonstration of long-term survival and functional integration into the adult CNS of grafted dopaminergic neurons. Page 26, lines 30-34.

More importantly, the specification details how to use the claimed method of treatment and provides a working example (Example 5, pages 26-28). Yet, the Examiner asserts that there is no working example of treatment of any neurological disease in humans and that there is no direction in the specification on sustaining therapeutic effect derived from grafting neuronal cells

gene therapy and cell transplantation therapy in a novel manner and discloses successes achieved by the Applicants.

The method of the invention is more sophisticated than traditional gene therapy in that it combines cell replacement therapy with gene therapy, thereby overcoming many of the obstacles to traditional gene therapy. For example, according to the invention, delivery of the gene of interest is accomplished *ex vivo* via transduction protocols well known in the art, as discussed in the specification at least at page 15. In this manner, the problem of targeting the vector to the specific cell is eliminated. The Examiner also notes that a further problem with traditional gene therapy has been obtaining sustained expression of the gene. However, it is in fact known in the art that transformed neural stem cells do not exhibit this difficulty. As evidence, Applicants have appended hereto "Gene Therapy Approaches to Neurodegenerative Disease," Wartiovaara, Neural Notes, Vol. 5, Iss. 3, 2000, pp. 5-8, which is a review of a 1999 symposium. The Examiner is directed to page 5, column 2 which notes "(neural stem cells) are easily transduced in vitro by common gene transfer methods, and they express the genes of interest rapidly and for long periods of time."

Citing Fox, the Examiner goes on to conclude that gene therapy is unpredictable. Again, however, the invention is not properly characterized as exclusively "gene therapy" for the reasons discussed above. And although even as properly characterized, the art as a whole may be unpredictable, this factor alone does not preclude patentability when viewed in light of the factors weighing in favor of enablement: the relative skill of those in the art, the extensive knowledge of the etiology of neurologic disorders and the specific and extensive guidance provided in the specification for the preparation of transformed dopaminergic cells for therapeutic purposes, including working examples. Each of these factors are discussed below.

As the Examiner recognizes at page 6 of the Office Action, the relative skill of those in the art of recombinant DNA technology is high. As noted above, the transfection of cells prepared according to the invention with a gene of interest is well within the capability of the skilled artisan. Furthermore, the art is replete with knowledge of specific deficiencies in synthetic pathways leading to the various neurological disorders, as discussed in the specification at least at pages 14-15. The MPEP notes that "a patent need not teach, and preferably omits, what is well-known in the art." MPEP 2164.01. Therefore, the skilled artisan, armed with the teachings of the specification and knowledge of restorative gene products that can alleviate a

into a brain. The working model disclosed illustrates the method of treatment in adult female rats in which Parkinsonian pathology is mimicked by creating pharmacological lesions of the nitrostriatal dopaminergic pathway. The MPEP 2164.02 explains that an *in vivo* animal model constitutes a working example "if the art is such that a particular model is recognized as correlating to a specific condition" and that such animal models "should be accepted as correlating unless the examiner has evidence that the model does not correlate." The Examiner has not provided any such evidence, therefore, the Examiner's assertion that there is no working example in humans is not dispositive of whether the invention is enabled as claimed. To the contrary, the art recognizes the rat model used in the working example. For example, the Examiner is directed to Bjorklund et al., attached hereto, which states in Column 3, "there are well-characterized rodent and primate models of PD, and although these have a different etiology from the human disease, they nevertheless mimic its cardinal features, and they have repeatedly proved to have good predictive value with respect to effects of therapeutic interventions on symptoms in PD patients" (Bjorklund et al., "Cell replacement therapies for central nervous system disorders," Nature Neuroscience, Vol. 3, No. 6, June 2000). Indeed, Clarkson et al., attached hereto, states that the rat model was developed in 1970 and has since been widely recognized in the art as an excellent animal model of PD. Page 2428. (Clarkson et al., "Minireview: Development of Fetal Neural Transplantation as a Treatment for Parkinson's Disease," Life Sciences, Vol. 65, No. 23, pp. 2427-2437).

In conclusion, Applicants respectfully submit that the specification, in conjunction with the high level of skill in the art, fully enables the pending and newly presented claims directed to delivery of prepared transformed or untransformed neural cells prepared according to the method of the invention. Withdrawl of the rejection and notification to that effect is earnestly solicited.

The Rejection of claims 1-11

Similar to the rejections of claims 12-15, the Examiner has rejected claims 1-11 with regard to the type of precursor cell used. On page 8 of the Office Action, the Examiner states "the present claims are very broad," asserting that claim 1 encompasses proliferating and differentiating any precursor cells.

The Examiner asserts that "the specification provides no evidence that the culture method would work on any precursor cell, rather than just on CNS precursor cells." Applicants

respectfully traverse the rejection. However, to further prosecution of the Application, and not to acquiesce to Examiner's rejection, Claim 1 has been amended to recite precursor cells comprising CNS stem cells.

The Examiner also asserts that the specification provides no evidence that the precursor cells obtained from human embryonic tissue between embryonic weeks 5 and 8 would develop dopaminergic neuron cells in the culturing method taught. The Examiner concludes that using precursor cells from any source other than the ventral mesencephalon of E12 rat embryos would require undue experimentation. Applicants respectfully traverse.

The MPEP states that for a claimed genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art (in view of the level of skill, state of the art and the information in the specification) would expect the claimed genus could be used in that manner without undue experimentation. MPEP 2164.02. In this case, the claimed genus is CNS precursor cells, and a working example provides evidence that the invention works as taught for a species of CNS precursor cells. "Proof of enablement will be required for other members of the claimed genus only where adequate reasons are advanced by the examiner to establish that person skilled in the art could not use the genus as a whole without undue experimentation." MPEP 2164.02. The Examiner has not demonstrated that the skilled artisan could not use the genus as a whole in the method taught.

On the other hand, ample guidance is provided in the specification for using CNS precursor cells in the method of the invention. For example, the specification teaches that precursor cells should be obtained during a "sensitive period." Page 5, lines 28-31. The specification provides the appropriate sensitive period for both rat embryonic and human fetal tissue. Further, the specification at pages 5-11 teaches in great detail the procedures for culturing, proliferating and differentiating CNS stem cells. Those of skill in the art would readily ascertain that the methods of the invention are applicable for any CNS stem cell. Indeed, Wartiovaara notes that "Most of the biology of (neural stem cells) has been studied in animals, but there seems to be little difference across mammalian species, since human fetal NSCs are able to integrate and ameliorate rodent disease model neuronal phenotypes." Page 6, col. 1. Therefore, the Examiner's unsupported contention that the physical structures of human brains and rodent brains during fetal development is markedly different, is not relevant. What is relevant is the conserved nature of neural stem cells, which is recognized in the art.

For these reasons, Applicants respectfully submit that the specification, in conjunction with the high level of skill in the art, fully enables the pending and newly presented claims directed to a method of culturing CNS precursor cells to generate dopaminergic neuron cells. Withdrawl of the rejection and notification to that effect is earnestly solicited.

Summary

In summary, Applicants respectfully assert that for the above reasons, the pending and newly presented claims are enabled by the specification as required by 35 U.S.C. §112, first paragraph and withdrawl of the rejection is earnestly solicited.

**Rejection under 35 U.S.C. § 112, second paragraph**

The Examiner has rejected claims 1-16 as being indefinite. Claim 1 was rejected for lacking a step which clearly relates back to the preamble. In response, Applicants have amended claim 1 to recite "a reaggregation of dopaminergic neuron cells" in step b, thereby relating this step back to the preamble and overcoming the rejection.

The Examiner has rejected claim 12 for failing to set forth any steps in the method/process. Claim 12 has been cancelled. New claim 20 recites the subject matter of cancelled claim 12, but recites steps in the method claimed, thereby overcoming the rejection.

The Examiner has rejected claim 14 for lacking a step which clearly relates back to the preamble. In response, Applicants have amended claim 14 to recite "transforming neuronal precursor cells with a gene encoding said gene product," thereby relating this step back to the preamble and overcoming the rejection.

The Examiner has rejected claim 16 for failing to set forth any steps in the method/process. Claim 16 has been cancelled. New claim 24 recites the subject matter of cancelled claim 16, but recites steps in the method claimed, thereby overcoming the rejection.

Because Examiner's rejection of claims 1-16 (and the new claims insofar as the rejection may apply) has been addressed by the amendments delineated herein, withdrawl of the rejection is respectfully requested.

**Rejection under 35 U.S.C. § 102**

The Examiner has rejected claim 17 as being anticipated by Lee et al. (U.S. Pat. No. 5,792,900), Johe et al. (Genes & Development (1996) 10:3129-40) and Deloulme et al. (J. Neuroscience Research (1991) 29:499-509). Applicants respectfully traverse the rejection. However, to advance prosecution of the Application, and not to acquiesce to the rejection, Applicants have cancelled Claim 17 and included its limitations in Claim 18. The Examiner is thanked for acknowledgement that Claim 18 is free of prior art and is allowable if it includes the limitations of the base claim. These amendments have placed the claims in condition for allowance and notification to that effect is earnestly solicited.

## CONCLUSION

In conclusion, each of claims 1-11, 14, 15 and 18-24 are in condition for allowance. The Examiner is invited to contact Applicant's undersigned representative at the telephone number listed below, if the Examiner believes that doing so will expedite the prosecution of this patent application.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

Dated: December 28, 2001

By: _____
Melissa Jean Pytel
Reg. No. 41,512

**Version with Markings to Show Changes Made:**

The following paragraphs of the specification were amended.

At page 2, the paragraph beginning at line 22:

-- FIG. 5 is a graph showing the effect of ascorbic acid and [glutathion] glutathione on the number of TH-ir cells grown in a cell culture.--

At page 2, the paragraph beginning at line 24:

--FIG. 6 is graphs showing the effects of ascorbic acid (AA), dopamine, and D-acetyl [cystein] cysteine on the number of TH-ir cells grown in a cell culture.--

At page 8, the paragraph beginning at line 4:

-- As used herein, the term dopaminergic neuronal cells refers to those cells generally found in the region of the ventral midbrain (VM) known as the substantia nigra pars compacta that project to the striatum. The precursor cells are typically found near the midbrain/hindbrain junction of an intact brain. Dopaminergic neurons can be characterized by their secretion of dopamine as a neurotransmitter and high levels of expression of tyrosine [hyroxylase] hydroxylase (TH), an enzyme that catalyzes the rate limiting step in the biosynthesis of dopamine.--

At page 10, the paragraph beginning at line 9:

--Preferably, the differentiated media includes at least one of cAMP, forskolin, dopamine and ascorbic acid. Preferably, cAMP is present in a concentration from about 1μM to about 5 mM, more preferably about 10μM to about 1 Mm. Typically, differentiation media containing cAMP results in about a 50% to about 300% increase in the total number of differentiated neural cells from precursor cells. Most typically, an increase in tyrosine [hyroxylase] hydroxylase immunoreactive (TH-ir) cells, also called dopaminergic cells is observed. Preferably forskolin is present in the differentiation medium at a concentration from about 1 μM to about 100 μM, more preferably about 2 μM to about 10 μM. Typically, differentiation media containing forskolin results in about a 40% to about 150% increase in the total number of differentiated neural cells, particularly TH-ir cells. Preferably dopamine is present at a concentration from about 0.1 μM to

about 1 mM, more preferably about 1μM to about 1 mM to provide about a 300% to about 700% increase in the total number of TH-ir cells. The effects of cAMP, forskolin and dopamine appear to be additive.--

At page 16, the paragraph beginning at line 17:

--Tissue pieces were spun in a centrifuge at 209g for 5 minutes and mechanically triturated to a quasi single cell suspension in HBSS and counted. 5 ml of cell suspension at a concentration of $150 - 200 \times 10^3$ cells/ml was plated on a[10cm] 10 cm culture[dishes] dish containing DMEM/F12/N2 medium (Bottenstein, J.E. & Sato, G.H. Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc.Natl.Acad.Sci.USA 76, 514-517 (1979) (modified according to [()Johe, K.K., Hazel, T.G., Müller, T., Dugich-Djordjevic, M.M. & McKay, R.D.G. Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. Genes and Development 10, 3129-3140 (1996)] that had been previously coated with polyornithine (15ug/ml) and fibronectin (1ug/ml).--

At page 19, the paragraph beginning at line 5:

--Using general immunohistochemical procedures known to those of skill in the art, the immunoreactivity of the cells to TH was examined using TH polyclonal 1:500 (Pel Freeze). While no tyrosine[hyrdoxylase] hydroxylase (a rate limiting enzyme in the synthesis of dopamine) immunoreactive (TH-ir) cells could be detected in the tissue at the time of dissection, after 7 days of differentiation 18.4% +/- 5.1% of the total cell population was immunoreactive for TH. TH-ir cells were also immunoreactive for dopamine, dopamine transporter (DAT) and for TuJ1. The percentage of TH-ir cells is non-expanded cultures, grown without bFGF was 5.6 +/- 1.3%.--

At page 22, the paragraph beginning at line 19:

-- Lipid peroxydation is a colorimetric assay (OXIS BIOXYTECH® LPO-568™; Oxis International Inc.) and was used according to the specifications of the manufacturer. $H_20_2$ assay measures the conversion of 2',7' dicholorofluorescin diacetate (Molecular Probes d-399) into 2',7' dicholorofluorescin catalyzed by $H_20_2$. The assay was performed according to the specifications of the manufacturer. [Immuniohistochemistry]Immunohistochemistry for [oxidative]oxidative

stress markers by the DAB-[peroxydase]peroxidase technique was performed on cells differentiated in the presence or absence of ascorbic acid, glutathione or D-acetyl-[cystein]cysteine plus ebselin. The following antibodies were used:

| Epitope | Antibody Type | Catalogue No. | Company |
|---|---|---|---|
| Anti-hemoxygenase I | monoclonal AB | OSA-111 | Stressgen |
| Anti-8-hydroxyguanosine | monoclonal AB | 12501 | QED Bioscience Inc. |
| Anti-Nitrotyrosine | polyclonal (sheep) AB | 24312 | Oxis International Inc.-- |

At page 23, the heading at line 28:

--E. **Combination treatment with cAMP, ascorbic acid and [(DA)]dopamine--**

At page 23, the paragraph beginning at line 29:

--Combination treatment experiments were carried out with mesencephalic precursors expanded with bFGF for 11 days and differentiated in DMEM/F12/N2 medium in the presence or absence of ascorbic acid (100uM), dopamine (1nM-1mM) and cAMP (1mM). The data revealed that dopamine has only a very minor additional effect on the yield of dopaminergic neurons as compared to ascorbic acid treatment alone (see Figure 6). [C]cAMP and ascorbic acid have an additive effect on the yield of dopaminergic neurons from expanded precursors. Combination treatment of ascorbic acid and [(DA)]dopamine and of ascorbic acid and cAMP showed that [DA]dopamine contributes no significant additional effect to ascorbic acid treatment. The effects of ascorbic acid and cAMP appear additive.--

At pages 24-25, the paragraph beginning at page 24, line 32:

-- The differentiation medium for reaggregate cultures consisted of Neurobasal®/2%B27® (Gibco, Life Technologies) with or without fetal bovine serum (FBS; 10%, Gibco), glial derived neurotrophic factor (GDNF; 10ng/ml; Peprotech), brain derived neurotrophic factor (BDNF; 10ng/ml; Peprotech), neurotrophin 4/5 (NT4/5; 10ng/ml; Peprotech), SHH (2.5µg/ml; kindly provided by Dr. Thomas Muller, at the [Lab.]Laboratory of Molecular Biology, currently at the Max Delbrueck Univ. in Berlin; SHH is now commercially avail. fro R&D). [Supplementation of the differentiating medium (consisting of Nerobasal/B27) with 10% RBS led to a dramatic [?]]--

Claims 1, 14 and 18 were amended as follows.

1. (AMENDED) A method of generating a cell culture comprising dopaminergic neuron cells, said method comprising:

    a. proliferating precursor cells, said step of proliferating comprising:

        i. incubating a suspension of said precursor cells in a proliferating medium which includes basic fibroblast growth factor (bFGF) to form proliferated precursor cells; and

    b. differentiating said precursor cells, said step of differentiating comprising:

        i. incubating said precursor cells in an incubation vessel which contains differentiation medium in a manner effective to form a reaggregation of differentiated dopaminergic neuron cells that is not adhered to any surface of the incubation vessel, wherein the differentiation medium includes ascorbic acid;

    wherein said precursor cells comprise CNS stem cells.

14. (AMENDED) A method of introducing a gene product into a brain of a patient, comprising:

    A. transforming neuronal precursor cells with a gene encoding said gene product;

    B. culturing said transformed neuronal precursor cells according to claim 1 to form differentiated transformed neuronal cells; and

    C. administering said differentiated transformed neuronal cells to a patient in need thereof.

18. (AMENDED) A cell culture comprising about 80% to about 95% of a total cell population in the culture comprise differentiated neuronal cells and less than 5% of the total cell population comprises glial cells [The cell culture of claim 17] wherein the differentiated neuronal cells comprise dopaminergic cells.

Claims 20-24 are new.